RESPONSE OF THE MEADOW VOLE (MICROTUS PENNSYLVANICUS) TO EXPERIMENTAL INOCULATION WITH BORRELIA BURGDORFERI

Authors: Campbell, G. Douglas, Barker, Ian K., Johnson, Roger P., Shewen, Patricia E., McEwen, Scott A., et al.

Source: Journal of Wildlife Diseases, 30(3) : 408-416

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-30.3.408

Journal of Wildlife Diseases, 30(3), 1994, pp. 408–416

RESPONSE OF THE MEADOW VOLE (*MICROTUS PENNSYLVANICUS*) TO EXPERIMENTAL INOCULATION WITH *BORRELIA BURGDORFERI*

G. Douglas Campbell,[1] Ian K. Barker,[1] Roger P. Johnson,[2] Patricia E. Shewen,[3] Scott A. McEwen,[4] and Gordon A. Surgeoner[5]

[1] Canadian Cooperative Wildlife Health Centre, Ontario Region, Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada N1G 2W1
[2] Health of Animals Laboratory, Agriculture Canada, Guelph, Ontario, Canada N1G 3W4
[3] Department of Veterinary Microbiology and Immunology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada N1G 2W1
[4] Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada N1G 2W1
[5] Department of Environmental Biology, Ontario Agricultural College, University of Guelph, Guelph, Ontario, Canada N1G 2W1

ABSTRACT: The response of the meadow vole (*Microtus pennsylvanicus*) to infection by experimental inoculation with *Borrelia burgdorferi* was evaluated. Forty-two adult voles were inoculated subcutaneously with 0.5×10^6 spirochetes. Sera taken during the 196 day trial were tested by indirect fluorescent antibody (IFA) assay for antibodies to *B. burgdorferi*. Tissues from animals which died during the trial, and from animals killed at 28, 112 and 196 days post-inoculation (DPI), respectively, were cultured in BSK-II medium for ≤6 weeks. They also were examined histologically for lesions and the presence of spirochetes. All inoculated animals developed antibodies by 14 DPI and maintained titers ≥1:10 for the duration of the trial. Spirochetes were isolated from ears, bladder, and spleen. Spirochetes also were identified by Bosma-Steiner silver stain or tissue IFA assay in sections of ears, bladder, kidney and heart. Infection as confirmed by re-isolation persisted for ≤111 days. No lesions were identified in association with the presence of spirochetes. No increase in mortality was observed in inoculated animals compared with controls.

Sensitivity of the IFA test at a cut-off titer of 1:10 was 100% from ≥14 DPI, but at 1:20 reached a maxiumum of 97%. Specificity at 1:10 was 84% and at 1:20 was 97%. Use of antiserum to *Microtus* immunoglobulin (Ig) in a double-layered test provided no significant advantages over use of a commercial fluorescein-conjugated anti-mouse Ig in a single-layered IFA test.

Key words: *Microtus pennsylvanicus*, meadow vole, *Borrelia burgdorferi*, Lyme borreliosis, experimental study, pathology, serology.

INTRODUCTION

Borrelia burgdorferi is a spirochetal bacterium which causes Lyme borreliosis, a multisystemic illness in humans (Steere, 1989). It is transmitted among wildlife reservoirs (Anderson, 1989) and to humans by the bite of a vector tick, usually of the *Ixodes ricinus* complex (Lane et al., 1991).

In eastern and midwestern North America, *I. scapularis* (Oliver et al., 1993) is the primary vector and the white-footed mouse (*Peromyscus leucopus*) is the principal reservoir host (Anderson, 1989). *Ixodes scapularis* also feeds upon a wide variety of small mammals other than *Peromyscus* sp., and *B. burgdorferi* has been recovered from many of these (Lane et al., 1991). Within grassy habitats in eastern North America, the meadow vole (*Microtus pennsylvanicus*) occurs abundantly and is infested with immature stages of *I. scapularis*, and can be infected with *B. burgdorferi* (Anderson et al., 1986). Its role as a reservoir host remains to be determined.

Our primary objective was to describe the response of *M. pennsylvanicus* to experimental infection with *B. burgdorferi*, including serological response to infection; localization of the bacterium by bacteriological isolation and by histological or histochemical identification; and evaluation of the host response to infection. Another objective was to evaluate the sensitivity and specificity of the indirect fluorescent antibody (IFA) test as a serological test for exposure to *B. burgdorferi* in this species.

MATERIALS AND METHODS

Meadow voles used in this study were from a laboratory colony maintained for 18 yr at the University of Guelph, Guelph, Ontario, Canada.

They had either been tested by the indirect fluorescent antibody (IFA) test for antibodies to *Borrelia burgdorferi* using the methods of Barker et al. (1992), or were the offspring of animals tested in this manner and found to be negative. Most were mature animals of 4 to 6 mo of age.

Thirty-two animals were assigned to an experimentally inoculated group and 16 to the non-inoculated control group; these animals were maintained for ≤196 days. Due to unexpected and unrelated mortality, 10 additional animals were inoculated experimentally 84 days after the first group. These animals were maintained for ≤112 days. Surviving animals from all groups were killed at the same time.

Inoculated animals were given a single subcutaneous injection of *B. burgdorferi* strain 231-LI3 originally isolated in 1987 from an engorged larval *I. scapularis* from Long Point, Ontario, Canada (42°30′N, 80°15′W). The identity of the isolate as *B. burgdorferi* was based on its reaction with monoclonal antibody H5332, courtesy of Dr. A. Barbour, University of Texas, San Antonio, Texas, USA. A frozen first passage culture, reisolated from an experimentally inoculated *Peromyscus leucopus*, was grown in BSK-II medium (Barbour, 1984) at 33 C, and spirochete numbers were estimated by counting under a dark field microscope a representative sample of the fields in 5 μl of the culture medium.

Following light anesthesia with isoflurane (AErrane, Anaquest, Mississauga, Ontario, Canada) animals were inoculated with an estimated total of 0.5×10^6 organisms in 0.5 ml of BSK-II medium, divided, and given subcutaneously at two sites in the dorsal aspect of the neck. Animals in the control group were similarly anesthetized, and each was inoculated similarly with 0.5 ml of sterile saline.

Following inoculation, animals were housed individually in shoebox style cages (20 × 32 × 23 cm) (Lab Products Inc., Maywood, New Jersey, USA). All animals experimentally inoculated with *B. burgdorferi* were housed within a single room in an isolation facility with double air-lock doors and a pressure gradient ventilation system. All control animals were housed in an identical manner in a similar separate room in the same facility. All animals were given water and a variety of rodent and dog chows *ad libitum*, supplemented periodically with fresh fruit and vegetables.

Six animals from the inoculated group were killed 28 days post-inoculation (DPI), and tissues were examined to confirm that the animals were successfully infected. Demonstration of spirochetes in tissue was attempted in several ways: culture of selected organs for bacterial isolation; histopathology using hematoxylin and eosin and silver impregnation stains; and immunofluorescent tissue staining. At the termination of the study all inoculated and control animals were killed and tissues were taken similarly for confirmation of infection and identification of associated lesions. Whenever possible, animals dying during the course of the experiment were examined similarly.

Serum samples were obtained prior to inoculation and at 7, 14, 21, 28, 56, 84, 112 DPI from all inoculated and control animals, and at 140, 168, and 196 DPI from only the inoculated group. The animals were anesthetized in a closed jar using either isoflurane or methoxyflurane (Metofane, Pitman-Moore Ltd., Mississauga, Ontario, Canada) and were bled from the retroorbital sinus using plain glass 250 μl capillary tubes (Fisher Scientific, Nepean, Ontario, Canada). Serum samples were diluted 1:5 in a solution of 5% bovine serum albumin (Sigma Chemicals, St. Louis, Missouri, USA) in phosphate buffered saline (PBS) and stored at −70 C until tested.

Tissue samples were obtained from the six inoculated animals killed at 28 DPI and from the remainder of the experimental and control animals at the termination of the study. The animals were deeply anesthetized with either isoflurane or methoxyflurane and were exsanguinated by cardiac puncture. The following tissue samples were obtained with aseptic techniques: spleen, kidney, bladder, and ears, and were placed into individual 60 ml sterile whirl pack bags (Fisher Scientific) containing 0.5 ml of BSK-II medium. Additional tissues from these organs, and samples of brain, heart, lung, liver and an entire hind limb were placed in 10% formalin for histological evaluation.

The tissue samples for bacterial culture were macerated within the bags and a 200 μl sample of the resulting tissue suspension was inoculated into 7 ml of BSK-II medium. The tubes were incubated at 33 C and examined using dark-field microscopy at 1, 3, and 6 wk for the presence of spirochetes. The estimated lower limit of detection using this isolation method was determined by performing serial dilutions of a culture and reinoculating 200 μl aliquots of the diluted culture into 7 ml tubes of medium; it was an estimated 10 organisms in the inoculum.

In addition to the animals killed at 28, 112, or 196 DPI, a number of animals died during the experiment. These animals were sampled as described, when conditions allowed. However, if post-mortem autolysis was advanced, no attempt was made to obtain tissues for histology, and only ears and bladder were cultured.

Antiserum to *Microtus* spp. immunoglobulin (Ig) was produced in rabbits by immunization with ammonium sulfate precipitated globulin (Harlow and Lane, 1988) derived from serum

pooled from *Microtus* spp. in the laboratory colony. The protein concentration of the isolated globulin fraction was estimated from the optical density measured on a spectrophotometer at 280 nm (Harlow and Lane, 1988). Two juvenile male New Zealand white rabbits (*Oryctolagus cuniculus*) (Maple Lane, Clifford, Ontario) each were inoculated with a mixture of 1.5 mg of *Microtus* spp. Ig and 100 μg of saponin adjuvant (Quil A, Cedarlane Laboratories Ltd., Hornby, Ontario). This inoculation was repeated twice at 3 wk intervals. Two weeks after the third injection, the rabbits were deeply sedated with an intramuscular injection of a mixture of 30 mg/kg ketamine (Ketaset, Ayerst Laboratories, Saint Laurent, Quebec, Canada) and 5 mg/kg xylazine (Rompun, Haver, Bayvet Division, Chemagro, Concord, Ontario) and were exsanguinated by cardiac puncture. The sera obtained were stored without further purification or preservatives in 250 μl aliquots at −70 C.

Microtus spp. serum was tested for antibodies to *B. burgdorferi* using a double-layered indirect fluorescent antibody (IFA) assay adapted as follows from the methods of Wilkinson (1984). The above antiserum to *Microtus* spp. Ig was employed as the second antibody, followed by fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG heavy and light chains (Kirkegaard & Perry Laboratories, Gaithersburg, Maryland, USA). Optimum concentrations of rabbit anti-*Microtus* spp. Ig and of FITC-conjugated goat anti-rabbit IgG producing optimal image contrast with minimal background were selected by checkerboard dilution in which a range of dilutions of each antisera were tested against each other. Serum from an experimentally infected vole from which the organism had been reisolated served as a positive control. Multi-well glass slides (ICN Biologicals Inc., Horsham, Pennsylvania, USA) were coated with a whole cell of strain 35210 *Borrelia burgdorferi* (American Type Culture Collection, Rockville, Maryland) antigen preparation with the following modifications of Wilkinson's (1984) method. All incubations were done at 37 C in a humidified chamber. Non-specific binding was blocked with a 10% solution of normal goat serum in PBS. Immediately following this step, the wells were incubated with test serum. Sera from inoculated animals were tested at doubling dilutions from 1:10 to 1:320 in PBS. Serum from control animals was screened at 1:10 and 1:20. After three washes in PBS, the slides were incubated with rabbit anti-*Microtus* spp. serum, diluted 1:50. The slides were washed again and then incubated with FITC-labeled goat anti-rabbit IgG diluted 1:100 in a 0.005% solution of Evan's Blue dye (Sigma Chemical Company) in PBS. Following a final wash, the slides were air dried and covered with coverslips with a 10% solution of glycerol in PBS as mounting medium.

Slides were examined immediately or stored in the dark at −20 C for ≤1 day before examination. The slides were examined under ultraviolet light using a Zeiss Standard 16 (Carl Zeiss Canada, Don Mills, Ontario) epifluorescence microscope, with the examiner unaware of the identity of the serum being read. Wells were scanned at 250×, and were scored as positive if ≥50% of the spirochetes present had specific fluorescence. Titers were reported as the highest dilution scored positive by this criterion. Positive and negative control sera from animals of known infection status were included with each batch of slides. To perform statistical tests, titers were transformed to $\log_2$ as described by Thrushfield (1986).

The sensitivity and specificity of the IFA test for determining exposure to *B. burgdorferi* were determined as described by Martin (1977). Using conventional terminology, disease-positive (exposed) animals were those which had been experimentally inoculated. Disease-negative (non-exposed) animals comprised both control and inoculated groups prior to inoculation. Test-positive animals (seroreactors) were those whose IFA titers reached or exceeded a specified cut-off titer; in this study, cut-off titers were 1:10, 1:20, or 1:40. The sensitivity, or proportion of test positives among sera from exposed animals, was calculated for each sampling period. The specificity, or proportion of test negatives among sera from non-exposed animals, was calculated using the pre-inoculation samples of both inoculated and non-inoculated control groups.

A subset of serum samples from each group, representing a range of titers and sampling dates, was retested using a single-layer IFA test employing FITC-labeled goat anti-mouse IgG & IgM (Kirkegaard & Perry Laboratories), at a dilution of 1:50, as the second antibody. Twenty-four serum samples obtained from inoculated animals at 21, 28, 56 or 84 DPI, and 25 pre-inoculation samples, were titrated in both double-layered (rabbit anti-*Microtus* spp. + goat anti-rabbit FITC conjugate) and single-layered (goat anti-mouse FITC conjugate) IFA tests. Results of this subset of sera were used to compare the tests as follows. The specificities of the two tests at cut-off titers of 1:10 and 1:20 were calculated and compared (Martin, 1977). The correlation coefficient of the log-transformed titers measured with double-layered and single-layered tests was calculated (Kirkwood, 1988). The significance of difference of the geometric means of the values obtained with the two tests was calculated with a paired *t*-test (Kirkwood, 1988). Using titers of 1:10 and 1:20 to classify the serum

samples as reactive or non-reactive, the degree of agreement between the two tests was estimated using a kappa statistic (Sackett et al., 1985).

The following tissues, when available and in condition suitable for testing, were examined microscopically: liver, spleen, kidney, urinary bladder, ears, heart, lung, and brain. Formalin-fixed tissues were embedded in paraffin and 5 μm sections were cut and stained with hematoxylin and eosin using the methods of Luna (1968). Slides from experimental and control animals were selected and examined randomly with the examiner unaware of their identity. The statistical significance of differences in the proportions of inoculated and control animals in which specific lesions were detected was tested using Fisher's exact test (Kirkwood, 1988).

Tissue sections 4 to 5 μm thick from all experimentally infected animals and from a subset of control animals were evaluated by the Bosma-Steiner method of silver impregnation (Dekoning et al., 1987).

Polyclonal antiserum for use in an indirect fluorescent antibody test on fixed tissue was raised against formalin-fixed *B. burgdorferi* in a rabbit. A first passage isolate of strain 231 LI3 was used as antigen. A young male New Zealand white rabbit was inoculated subcutaneously with 1 ml of a suspension of bacteria killed in 10% formalin and washed in PBS, mixed with 100 μg of saponin adjuvant. This dose was repeated twice more at three week intervals. Serum obtained three weeks after the last inoculation of antigen had a titer of 1:1,280 when examined by IFA test for antibodies to *B. burgdorferi*.

The immunofluorescent staining method used was the following modification of an immunoperoxidase method (Haines and Chelack, 1991). Following rehydration, tissue sections were incubated for 30 min with 0.05% Protease Type XIV (Sigma Chemicals Co.), washed three times in PBS, blocked with 10% normal goat serum in PBS, and then incubated overnight at 4 C with rabbit polyclonal anti-*Borrelia* serum diluted 1:50 in PBS. Following a second wash, the sections were incubated for 30 min with FITC-conjugated goat anti-rabbit IgG heavy and light chains diluted 1:100 in a 0.005% solution of Evan's Blue dye in PBS. Following a final wash, coverslips were mounted with 10% glycerol in PBS.

Slides were examined under ultraviolet light with a Zeiss Standard 16 epifluorescence microscope with the examiner unaware of the identity of the specimen. Spirochetes were identified as structures of size and morphology typical of *B. burgdorferi* showing specific fluorescence. Smears of spirochete cultures used as antigen in the serum IFA test served as positive controls. Tissues from experimentally inoculated animals incubated with normal rabbit serum instead of the polyclonal antiserum, and tissues from non-inoculated control animals incubated with the polyclonal antiserum, served as negative controls.

RESULTS

A number of animals died of apparent anesthetic overdose, and other spontaneous mortalities occurred at a similar rate in both inoculated and control groups. A post-mortem diagnosis was made in only a few instances. Many animals showed non-specific clinical signs such as ruffled hair coat and inappetence and at necropsy were in poor body condition with no detectable lesions. No clinical signs of neurologic or musculoskeletal disease were apparent.

Infection was confirmed by bacterial isolation in four of six inoculated animals sampled <30 DPI. During all phases of infection, spirochetes were most consistently isolated from the ears (13 of 30 animals), with the urinary bladder next in frequency of isolation (three of 22). The single isolates from each of kidney and spleen were from animals dying <30 DPI.

The longest duration of infection confirmed by isolation was 111 days. Five of the isolates from ears were from animals which had died unexpectedly and were not necropsied while fresh; 2 had been refrigerated for as long as 96 hours. At the termination of the study, spirochetes were not isolated from any tissues of the three surviving inoculated voles, nor from tissues of control animals.

All inoculated animals produced anti-*Borrelia* antibodies, as detected with rabbit anti-*Microtus* Ig in the double-layered IFA test; titers were ≥1:10 by 14 DPI. Inoculated animals never were seronegative (titer < 1:10) during the study period (Fig. 1), but 15 of 216 samples from inoculated animals tested had titers of 1:10. Most samples from control animals had titers <1:10; however, 15 of 88 samples, representing 11 of 16 control animals, had titers of 1:10. Sensitivity at a cut-off titer of 1:10 was 100% from 14 DPI onwards,

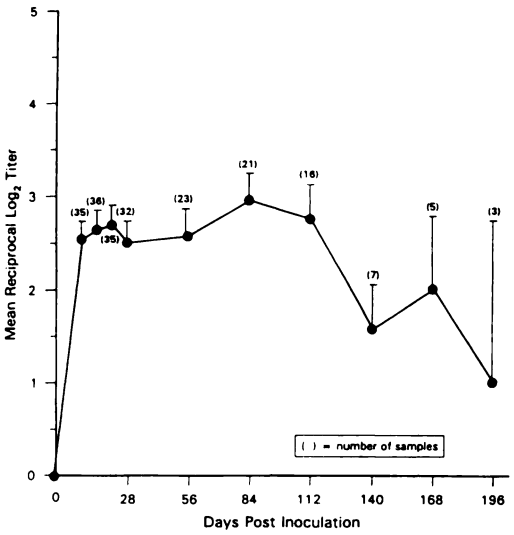

FIGURE 1. Means and standard errors of reciprocal $\log_2$ IFA titers of sera, starting dilution 1:10, from *Microtus pennsylvanicus* experimentally inoculated with *Borrelia burgdorferi*.

and at 1:20 reached a maximum of 97% (Table 1). Specificity at a cut-off titer of 1:10 was only 84% (49 of 58 samples), and was 97% (56 of 58 samples) at 1:20.

In comparing the results of IFA assay in the subset of sera from animals of known positive and negative status, the correlation coefficient for the single-layered and double-layered tests was 0.92. There also was a high degree of agreement between the two tests in classifying sera as reactors or non-reactors, as indicated by a kappa of 0.79 at a cut-off titer of 1:10 and of 0.92 at 1:20. There was no significant difference ($P \geq 0.05$) between titers obtained using the two tests. Sensitivity of the test using anti-*Microtus* spp. Ig was 100% at 1:10, and 96% at 1:20, while sensitivity with anti-mouse Ig was 100% at both cut-off titers. The specificity of the test using anti-*Microtus* spp. Ig was 96% at 1:10 and 100% at 1:20; using anti-mouse Ig it was 94% and 98%, respectively.

TABLE 1. Sensitivity of indirect fluorescent antibody (IFA) assay for detection of exposure to *Borrelia burgdorferi* in experimentally inoculated *Microtus pennsylvanicus* using cut-off titers of 1:10 to 1:40, at various days post-inoculation (DPI).

	Cut-off titer					
	≥1:10		≥1:20		≥1:40	
DPI	Test positives*	Sensitivity (%)	Test positives	Sensitivity (%)	Test positives	Sensitivity (%)
7	35/38	92	34/38	89	28/38	74
14	36/36	100	35/36	97	28/36	78
21	35/35	100	34/35	97	29/35	83
28	32/32	100	28/32	88	27/32	84
56	23/23	100	21/23	91	18/23	78
84	21/21	100	20/21	95	18/21	86
112	16/16	100	15/16	94	14/16	88
140	7/7	100	6/7	86	3/7	43
168	5/5	100	4/5	80	4/5	80
196	3/3	100	1/3	33	1/3	33

* Number positive/number examined.

Microscopic lesions commonly seen in hematoxylin and eosin stained sections from both inoculated and control animals included glomerulonephropathy, adrenal cortical hypertrophy, and periacinar hepatocellular vacuolation and necrosis. Other lesions were present only in a small number of inoculated animals. A mononuclear infiltrate in the myocardial interstitium was present in four of 20 animals. This infiltrate varied from mild and diffuse to a multifocal myocarditis in one animal. Mild, multifocal dermal mononuclear infiltrates were observed in the ears of four of 14 inoculated animals. One of six bladders from inoculated animals contained a similar submucosal infiltrate. There never was a statistically significant difference in the proportion of inoculated and control animals in which a lesion was detected and no spirochetes were detected in association with these lesions.

In tissues examined by IFA, small numbers of spirochetes were seen in the ears of five of 19 animals and in the bladder of one of 8 animals. The Bosma-Steiner silver stain produced the best images of spirochetes in tissue, but again, only low numbers were seen in a few of the inoculated animals. Using this technique, organisms were most commonly seen in the ears (six of 20 animals), and also were detected in one of 20 bladders, one of 20 kidneys and one of 21 hearts.

TABLE 2. Confirmation of infection with *B. burgdorferi* in experimentally inoculated *Microtus pennsylvanicus*: comparison of bacterial culture, Bosma-Steiner silver stain, tissue indirect fluorescent antibody (IFA) test, and serum IFA titer at time of death or at the sampling period prior to death.

Animal no.	DPI[a]	Culture	Silver stain	Tissue IFA	IFA titer
1	9	ears[b]			
2	23	ears, bladder, kidney	ears		40
3	27	ears, bladder	ears	ears	20
4	28	ears	kidney, heart		80
5	28	ears, bladder			40
6	28	ears, spleen			80
7	30		ears	ears	80
8	32	ears			40
9	34	ears			40
10	59	ears			160
11	61	ears			80
12	86	ears			80
13	104	ears			160
14	111	ears			40
15	112		ears, bladder, kidney		320
16	112		bladder	bladder, ears	160
17	114		ears		40
18	114			ears	80
19	196			ears	10

[a] DPI, days post inoculation.
[b] Tissue in which *B. burgdorferi* was demonstrated.

Using either staining method, spirochetes were seen only rarely, and a large number of high power (40×) fields were searched for each occurrence. Usually a single organism was detected, although rarely two or three organisms were seen in a single field. There was no associated inflammatory response. Spirochetes were identified in the dermis of ears, at all levels of the bladder wall, and in the intertubular interstitium of kidneys around arteries. In one animal, spirochetes were identified in the lumen of large blood vessels and in the adjacent interstitium of the heart.

Borrelia burgdorferi infection was confirmed at death in 19 of 42 inoculated animals (Table 2). One animal was confirmed infected by all three methods. Two animals were confirmed by culture and silver staining, and two by silver staining and tissue IFA staining. Ten animals were confirmed by culture alone, and two each by tissue IFA or silver staining alone. Using a cut-off titer of 1:20 in the IFA test, 18 of 19 of these animals had serological evidence of infection at the time of death.

DISCUSSION

Microtus pennsylvanicus developed a multisystemic infection in response to inoculation with *B. burgdorferi*. This was supported by bacterial isolation ≤111 DPI and by tissue staining ≤196 DPI. No evidence of disease was associated with this infection. The rate of occurrence of spontaneous deaths of unknown etiology and the occurrence of microscopic lesions were similar in both inoculated and control groups, making it highly unlikely that either could be attributed to infection with *B. burgdorferi*. Sporadic mortalities of undetermined cause have occurred historically in this colony and while these events were logistically unfortunate, there was no evidence associating them with *B. burgdorferi* infection.

The reservoir potential of *M. pennsylvanicus* was inferred by its ability to maintain an infection with *B. burgdorferi* over a period of time equal to the annual period of inactivity of vector ticks, without the occurrence of significant disease or mortality as the result of infection. The observed persistent infection (≤196 days), in spite of a detectable antibody response, is evidence that *M. pennsylvanicus* may be capable of acting as a reservoir of *B. burgdorferi* infection for tick vectors.

Spirochetes persisted in tissue without inciting an inflammatory response, as has been reported in *P. leucopus*, a known reservoir (Wright and Nielsen, 1990). In species such as humans, SCID mice and juvenile Lewis rats, in which infection with *B. burgdorferi* results in disease, similarly low numbers of organisms are seen in tissue sections (Duray, 1989; Barthold, 1991), but a marked inflammatory response occurs. Much of the tissue damage apparently results from inflammatory mediators released by the host (Szczepanski and Benach, 1991). How the organism persists in the tissues of rodent reservoirs in the presence of antibody without evoking inflammation and tissue damage is not known (Szczepanski and Benach, 1991).

Spirochetes were isolated from the tissues of animals that had been dead for ≤96 hours. Based on the persistence of spirochetes in tissue, we propose that cannibalism, a behavior which occurs in rodents, might provide an alternative means of transmission of *B. burgdorferi* within vole populations. In *Peromyscus*, oral transmission of *B. burgdorferi* is possible (Burgess and Patrican, 1987) and some form of oral transmission, whether by cannibalism or urine-oral contact, might explain infections in voles in populations where no ticks were identified (Callister et al., 1991).

Although xenodiagnosis provides the most significant biological measure of reservoir competence (Burgdorfer and Gage, 1987; Mather et al., 1989) it was not done for logistical reasons. It might have provided confirmation of infection status in animals where bacterial isolation and tissue staining failed.

The absence of sensitive and reliable confirmatory tests for detection of infection with *B. burgdorferi* has resulted in reliance upon serology as a diagnostic tool in human medicine. Similarly, serological and epizootiologic studies are important in populations of wildlife reservoirs. Serum samples are easily obtained from wild-caught rodents, and testing of serum for antibodies to *B. burgdorferi* is simpler and less expensive than alternative methods of identifying infected animals, such as by the polymerase chain reaction, bacterial isolation, or histological examination of tissues.

Since the performance characteristics of a serological test are difficult to determine in an observational study, the sensitivity and specificity of the IFA assay were determined using sera from animals of known infection status. Antibody to *B. burgdorferi* was detectable by IFA test at 7 DPI and at a dilution of 1:10 remained detectable in the three animals who lived the full 196 days of the study. Sensitivity, using a cut-off titer of 1:20, reached 95% by 84 DPI, and began to decline beyond 140 DPI. In this study of *Microtus* sp., using a cut-off titer of 1:20, the test has a specificity of 97%. The performance of serologic tests was not appreciably improved by the use of species-specific anti-*Microtus* spp. Ig in a double-layered test; use of anti-mouse Ig FITC conjugate in a single-layered test was an acceptable alternative.

Based on these results, the IFA test appears to be an appropriate screening test for epizootiological studies detecting exposure to *B. burgdorferi* in a population of wild rodents. Early development of an antibody response, and persistence of this response for at least 7 mo make it likely that most exposed animals will be detected. There are reports of wild-caught *Peromyscus* spp. from which spirochetes were isolated but which were negative for antibodies by either IFA test or enzyme-linked immunosorbent assay (ELISA) or

both (Magnarelli et al., 1988; Barker et al., 1992). These animals may have been in the acute (<7 days) stage of infection when the organism was more likely to be recovered, but before an antibody response had developed.

Specificity is the most significant limitation of the IFA test against *B. burgdorferi*. There is some cross-reactivity with antibodies against other spirochetes, particularly other *Borrelia* spp. and *Treponema* spp. (Magnarelli et al., 1987, 1990; Luther and Moskophidis, 1990), and with antibodies to antigens shared among an even wider range of bacterial species (Hansen et al., 1988; Coleman and Benach, 1992). The specificity as reported here may be higher than in field populations. As a laboratory colony, maintained in isolation, these animals probably would not have been exposed to as wide of a range of bacteria as would animals living under natural conditions. In field-caught rodents, one would expect a greater number of cross-reactions, and hence a higher proportion of false-positive results.

Even with the specificity as measured here, there are limitations to the use of the IFA in epizootiological studies. Antibody surveys of rodents often will be conducted in areas where *B. burgdorferi* has not been detected, and if present, is likely to occur at a low prevalence. Under these conditions, a significant number of test-positive animals will be false positives, and the predictive value of a positive test will be correspondingly low (Hart, 1983). For serologic testing in areas of low prevalence, it is desirable to retest positive sera using a test of higher specificity, such as immunoblotting.

Microtus pennsylvanicus appears capable of acting as a reservoir for *B. burgdorferi*. The IFA test is suitable for screening populations of this species for antibodies to that agent. The single-layered test using commercial anti-mouse Ig FITC conjugate is as effective as the double-layered test with anti-*Microtus* antiserum for assay of serum antibodies to *B. burgdorferi*.

ACKNOWLEDGMENTS

Technical assistance was provided by Laurie Elliott, Jan Kolar, Gareth Jones and Jackie Rombeek. Dr. L. Fromm provided facilities for silver staining at the histology laboratory, Department of Pathology, Women's College Hospital, Toronto, Canada. Dr. Alan Barbour, University of Texas Health Center, San Antonio, Texas, USA, kindly provided monoclonal antibody H5332. This work was supported by the University of Guelph Research Enhancement Fund, by the Ontario Ministry of Health, and by Health and Welfare Canada.

LITERATURE CITED

ANDERSON, J. F. 1989. Epizootiology of *Borrelia* in *Ixodes* tick vectors and reservoir hosts. Reviews of Infectious Diseases 11: S1451–1459.

———, R. C. JOHNSON, L. A. MAGNARELLI, F. W. HYDE, AND J. E. MYERS. 1986. *Peromyscus leucopus* and *Microtus pennsylvanicus* simultaneously infected with *Borrelia burgdorferi* and *Babesia microti*. Journal of Clinical Microbiology 23: 135–137.

BARBOUR, A. G. 1984. Isolation and cultivation of Lyme disease spirochetes. The Yale Journal of Biology and Medicine 57: 521–525.

BARKER, I. K., G. A. SURGEONER, H. ARTSOB, S. A. MCEWEN, L. A. ELLIOTT, G. D. CAMPBELL, AND J. T. ROBINSON. 1992. Distribution of the Lyme disease vector, *Ixodes dammini* (Acari: Ixodidae) and isolation of *Borrelia burgdorferi* in Ontario. Journal of Medical Entomology 29: 1011–1022.

BARTHOLD, S. W. 1991. Infectivity of *Borrelia burgdorferi* relative to route of inoculation and genotype in laboratory mice. The Journal of Infectious Diseases 163: 419–420.

BURGDORFER, W., AND K. L. GAGE. 1987. Susceptibility of the hispid cotton rat (*Sigmodon hispidus*) to the Lyme disease spirochete (*Borrelia burgdorferi*). American Journal of Tropical Medicine and Hygiene 37: 624–628.

BURGESS, E. C., AND L. A. PATRICAN. 1987. Oral infection of *Peromyscus maniculatus* with *Borrelia burgdorferi* and subsequent transmission by *Ixodes dammini*. American Journal of Tropical Medicine and Hygiene 36: 402–407.

CALLISTER, S. M., J. A. NELSON, R. F. SCHELL, D. A. JOBE, R. BAUTZ, W. A. AGGER, AND J. COGGINS. 1991. Survey for *Ixodes* spp. and *Borrelia burgdorferi* in southeastern Wisconsin and northeastern Illinois. Journal of Clinical Microbiology 29: 403–406.

COLEMAN, J. L., AND J. L. BENACH. 1992. Characterization of antigenic determinants of *Borrelia burgdorferi* shared by other bacteria. The Journal of Infectious Diseases 165: 658–666.

DEKONING, J., R. B. BOSMA, AND J. A. A. HOOGKAMP-KORSTANJE. 1987. Demonstration

of spirochaetes in patients with Lyme disease with a modified silver stain. Journal of Medical Microbiology 23: 261–267.

DURAY, P. H. 1989. Clinical pathologic correlations of Lyme disease. Reviews of Infectious Diseases 11: S1487–1493.

HAINES, D. M., AND B. J. CHELACK. 1991. Technical considerations for developing enzyme immunohistochemical staining procedures on formalin-fixed paraffin-embedded tissues for diagnostic pathology. Journal of Veterinary Diagnostic Investigation 3: 101–112.

HANSEN, K., J. M. BANGSBORG, H. FJORDVANG, N. S. PEDERSEN, AND P. HINDERSSON. 1988. Immunochemical characterization of and isolation of the gene for a *Borrelia burgdorferi* immunodominant 60-kilodalton antigen common to a wide range of bacteria. Infection and Immunity 56: 2047–2053.

HARLOW, E., AND D. LANE. 1988. Antibodies: A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, New York, pp. 100–104, 114–119, 288–299.

HART, G. 1983. Predictive value of serologic tests. American Journal of Public Health 73: 1288–1292.

KIRKWOOD, B. R. 1988. Essentials of medical statistics. Blackwell Scientific Publications, Oxford, England, pp. 41–45, 57–64.

LANE, R. S., J. PIESMAN, AND W. BURGDORFER. 1991. Lyme borreliosis: Relation of its causative agent to its vectors and hosts in North America and Europe. Annual Review of Entomology 36: 587–609.

LUNA, L. G. (editor). 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology, 3rd ed. McGraw-Hill, New York, New York, pp. 235–236.

LUTHER, B., AND M. MOSKOPHIDIS. 1990. Antigenic cross-reactivity between *Borrelia burgdorferi*, *Borrelia recurrentis*, *Treponema pallidum*, and *Treponema phagedenis*. Zentralblatt für Bakteriologie und Hygiene 274: 214–226.

MAGNARELLI, L. A., J. F. ANDERSON, AND R. C. JOHNSON. 1987. Cross-reactivity in serological tests for Lyme disease and other spirochetal infections. The Journal of Infectious Diseases 156: 183–188.

———, ———, K. E. HYLAND, D. FISH, AND J. B. MCANINCH. 1988. Serologic analyses of *Peromyscus leucopus*, a rodent reservoir for *Borrelia burgdorferi*, in northeastern United States. Journal of Clinical Microbiology 26: 1138–1141.

———, J. N. MILLER, J. F. ANDERSON, AND G. R. RIVIERE. 1990. Cross-reactivity of nonspecific treponemal antibody in serologic tests for Lyme disease. Journal of Clinical Microbiology 28: 1276–1279.

MARTIN, S. W. 1977. The evaluation of tests. Canadian Journal of Comparative Medicine 41: 19–25.

MATHER, T. N., M. L. WILSON, S. I. MOORE, J. M. C. RIBEIRO, AND A. SPIELMAN. 1989. Comparing the relative potential of rodents as reservoirs of the Lyme disease spirochete (*Borrelia burgdorferi*). American Journal of Epidemiology 130: 143–150.

OLIVER, J. H., JR., M. R. OWSLEY, H. J. HUTCHESON, A. M. JAMES, C. CHEN, W. S. IRBY, E. M. DOTSON, AND D. K. MCCLAIN. 1993. Conspecificity of the ticks *Ixodes scapularis* and *I. dammini* (Acari: Ixodidae). Journal of Medical Entomology 30: 54–63.

SACKETT, D. L., R. B. HAYNES, AND P. TUGWELL. 1985. Clinical epidemiology: A basic science for clinical medicine. Little, Brown and Company, Boston, Massachusetts, pp. 27–31.

STEERE, A. C. 1989. Lyme disease. New England Journal of Medicine 321: 586–596.

SZCZEPANSKI, A., AND J. L. BENACH. 1991. Lyme borreliosis: Host responses to *Borrelia burgdorferi*. Microbiological Reviews 55: 21–34.

THRUSHFIELD, M. 1986. Veterinary epidemiology. Butterworths, London, England, pp. 175–186.

WILKINSON, H. W. 1984. Immunodiagnostic tests for Lyme disease. Yale Journal of Biology and Medicine 57: 567–572.

WRIGHT, S. D., AND S. W. NIELSEN. 1990. Experimental infection of the white-footed mouse with *Borrelia burgdorferi*. American Journal of Veterinary Research 51: 1980–1987.

Received for publication 19 February 1993.